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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF THE MEDICINAL PLANTS *Andrographis paniculata* AND *Melia Azadirach L.*

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ABSTRACT

In the present investigation antimicrobial activity of leaf extracts and various column eluted fractions of the medicinal plants *Andrographis paniculata* and *Melia azadirach L.* were evaluated against the clinical bacterial strains *viz. Pseudomonas fluorescence, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus* and the *fungal strains viz., Trichophyton rubrum, Aspergillus niger, Aspergillus fumigatus and candida albicans.* The antimicrobial activity was carried out by agar disc diffusion and well cut methods. The methanol extracts of *Andrographis paniculata* and *Melia azadirach L* were found with potential antimicrobial activity against *Pseudomonas aueruginosa* (22mm), *Staphylococcus aureus* (21mm) and moderate activity was recorded against *Vibrio cholerae* (14mm).

INTRODUCTION

Plants have been an essential part of human society since the cultivation. The herb known as *Andrographis paniculata* is found growing in the forests of Asian countries such as China, India, Pakistan and Thailand. It is an erect and branched herb mostly seen in the wild. Traditional practitioners use this plant to treat hyperdipsia, wounds, ulcers, chronic fever, malarial and cough, bronchitis, skin diseases, leprosy, flatulence, colic, diarrhoea, dysentery (Warrier *et al.,* 1993). The use of *Andrographis* during recent researches produced favorable results, these observations during the particular study suggests that the *Andrographis* herb possesses very potent cell and antiulcerogenic effects of Andrograpolide (Madav *et al.,* 1995). The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites such as alkaloids, steroids, tannis, and phenolic compounds, flavonoids, resins fatty acids gums which are capable of producing definite physiological action on body (Joshi *et al.,* 2009). In Thailand, this plant was selected by the Ministry of Public Health as one of the medicinal plants to be included in "The National List of Essential Drugs A.D. 1999" (List of Herbal Medicinal Products) (Pholphana *et al.,* 2004; Kanokwan and Nobuo, 2008). The risk of being affected by the common cold was reduced by fifty percent in all patients who took 200 mg every day of *Andrographis paniculata* preparation –commonly marketed as Kan Jang, these doses were carried out throughout the duration of the cold season when common colds typically tend to affect susceptible individuals (Burgos, *et al.,* 1994). It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases (Mishra *et al.,* 2007). In Scandinavian countries, it is commonly used to prevent and treat common cold (Caceres *et al.,* 1997). *Melia azedarach* L. (Meliaceae) one of the another medicinal plants is commonly known as Mahanimba, Ramyaka, Dreka, Karmuka, Keshamushti, Persian lilac and White cedar. *M. azedarach* L. is a native of West Asia and now naturalized throughout the warm countries. In India, it grows wild in the Sub - Himalayan tract up to 1800 m. The various parts of the tree are reputed to have the same therapeutic values as those of neem tree **(**Stinson *et al*., 2003). Numerous reports are available on the pharmaceutical potentials of *Melia azedarach* L. Wide range of biological activities including diuretic activity, antihypertensive, anti-inflammatory, antibacterial, antifungal,hepto-protective, antioxidant, antitumor, anti-allergic, and antimalarial activity (Banskota *et al*., 2003 and Bayaty *et al*., 2010). In this present research an attempt was made to explore the antimicrobial potential of methanol extract and various coloumn eluted fractions of the medicinal plants against the clinical pathogens bacterial strains.

MATERIALS AND METHODS

The leaves of the medicinal plant *Andrographis paniculata* and *Melia azadirach L.* were collected from Western Ghats,

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Pavanasam, Tirunelveli District, and Tamilnadu. The leaves were dried under shade condition and ground into powder by using electrical grinder. The extraction of powdered leaves were achieved by Soxhlet apparatus. The extracts were dried and dissolved in DMSO solution (Dimethyl sulfoxide 100 µg/ml) and screened for antimicrobial activity against the clinical bacterial and fungal pathogens.

Phytochemical screening

The preliminary phytochemical analysis was performed as per the method of Kokate, (1993).

Detection of Triterpenoids

To the filtrate of extract one or two pieces of tin and three drops of thionyl chloride are added slowly, a violet or purple colour formation was recorded which indicates the presence of triterpenoids.

Detection of reducing sugars

Fheling's Test

Small portion of the various filtrates were treated with Fehling's solutions I and II heated on a water bathe and was observed for the formation of a brick red color.

Benedict's test

Small portion of the various filtrates were treated with equal quantities of Benedict's reagent and observed for the formation of a yellow colored precipitate.

Detection of Alkaloids

Small fractions of various filtrates were sepatately stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloid reagents such as Mayer's, and Dragendorff's reagent.

Mayer's Test

To small quantity of the various filtrates Mayer's reagent was added and observed for the formation of cream colored precipitate.

Hager's Test

To small quantity of the various filtrates, add Hager's reagents. Formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

To small quantity of the various filtrates, add Wagner's reagent. Formation of reddish brown coloured precipitate indicates the presence of alkaloids.

Dragendroff's Test

To small quantity of the various filtrates, add Dragendroff's reagents. Formation of reddish brown coloured precipitate indicates the presence of alkaloids.

Detection of phenolic compounds

Ferric chloride Test

To the filtrate few drops of neutral ferric chloride was added. The appearance of an intense blue or violet colour was recorded. This indicates the presence of phenolic compounds.

Detection of Saponins

Foam Test

Small portion of the various filtrates were diluted with 20 ml of distilled water and it was agitated in a cyclic mixer for 15 minutes. Formation of foamy layer was recorded; it indicates the presence of saponins.

Detection of Xantho proteins

To the filtrates, add few drops of concentrated Nitric acid with excess amount of Ammonia solution. Formation of Reddish orange coloured precipitate indicates the presence of Xantho proteins.

Detection of Tannins

Development of blue green color in the extract when treated with ferric chloride indicates the presence of tannin.

Detection of Aromatic acids

To small quantity of the various filtrates add saturated sodium bicarbonate, no brisk effervescence was noted, which indicates the presence of Aromatic acids.

Detection of Flavonoids

The extract is treated with few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids.

Detection of phytosterols

Small quantity of various filtrates were dissolved in 5ml of chloroform solution and was subjected to salkowski's and Liebermann-Burchard's test.

Salkowski's Test

To the 1ml of above prepared chloroform solution, few drops of concentrated sulfuric acid was added. It gave a red colour which indicates the presence of phytosterols.

Liebermann-Burchard's Test

The above prepared chloroform solution was treated with few drops of concentrated sulfuric acid and followed by treatment with 1ml of acetic anhydride solution. Formation of green colour, indicates the presence of phytosterol.

ANTIBACTERIAL AND ANTIFUNGAL ASSAY

Antimicrobial activity was determined by Agar well diffusion method (Shanab *et al*., 2004) with minor modifications. The microbial strains were procured from Microbial Type Culture

Collection (MTCC), Chandigarh, India. Bacterial strains *viz, Pseudomonas fluorescence (NCIM-2099), Vibrio cholerae (NCIM-1738), Escherichia coli (NCIM-(2065)), Klebsiella pneumoniae (NCIM-(2719), Salmonella typhi (NCIM-2263), Staphylococcus aureus (NCIM-5021) and four fungal strains Tricophyton rubrum (NCIM-1221), Aspergillus niger (1207), Aspergillus fumigates (NCIM-1811),* and *Candida albicans (NCIM-3471).*

The antimicrobial and antifungal activity was carried out by agar disc diffusion (Bauer *et al*., 1966) and well cut method. The sterile discs impregnated with 100 µl of *Andrographis paniculata and Melia azadirach.L* leaf extracts (100 µg/ml) were placed on the test pathogen seeded agar plates. The plates were kept for half an hour for pre incubation diffusion and later kept for incubation at 37^{0} C for 24hrs for bacteria and 48 hrs for fungal strains respectively. After the incubation time was over, the plates were observed for the measurement of inhibition zones produced by the extracts of these plants against the clinical microbes. The study was carried out in triplicate. Streptomycin disc (6mm) was used as a standard for bacterial cultures and flucanozole discs (6mm) for fungal strains.

TLC Technique

Each fractions of the column eluted sample was subjected to TLC to find out the separation of single compound.

Thin Layer Chromatography was performed on prepared plates with Silica gel F254 grade (Merck, Darmstadt, Germany) as stationary phase. A one-dimensional ascending development technique was used to detect the constituents of an extract on TLC plate solvent system like Ethyl acetate, methanol (16:22). Visual detection was done in daylight and under UV light at a wave length of 254 and 344 nm depending on the nature of compounds separated.

RESULTS AND DISCUSSION

The phytochemical analysis of the leaf extract of *Andrographis paniculata and Melia Azadirach.L were* tested *positive for* the presence of Triterpenoids, Reducing sugars, Tannins, Alkaloids, Flavonoids and Saponins (Table 1). Methanol extract of *A.paniculata* leaves showed potential antimicrobial activity against P.aueruginosa (22mm) and s.aureus (21mm), Methanol extract of *A.paniculata* have antimicrobial potential activity against two bacterial pathogens viz., *P.aeruginosa and s.aureus* (Pushpendra Kumar Mishra 2013; Gnan and Demello, 1999 and Fleisher, *et al*., 2003). The Methanol extracts of *Melia Azadirach.L,* also showed good inhibitory activity against Vibrio *cholera and Staphylococcus aureus* and the zone of inhibition recorded were18 mm and 14mm respectively for the above pathogens (Table 2). The antimicrobial activity may be due to the presence of Alkaloids, flavonoids and phenolic compounds present in the crude extracts. The extracts of

Table 1. Phytochemical constituents of *Andrographis paniculata* and *Melia azadirach L.* Leaf Extract.

Phytoconstituents	Methanol extract		Aqueous extract	
	Andrographis paniculata	*Melia azadirach L.*	*Andrographis paniculata*	*Melia azadirach L.*
Triterpenoids	+	+	_	_
Reducing sugars	+	_	_	_
Tannins	+	+	_	_
Triterpenoids	_	_	_	_
Phenolic compounds	_	_	+	_
Alkaloids	+	+	+	_
Flavonoids	+	+	_	_
Xantho proteins	_	_	_	_
Saponins	+	+	+	+

+ Indicates Positive _ Indicates negative

Table 2. Antimicrobial activity of methanol extract of *Andrographis paniculata* and *Melia azadirach.L* (Agar Disc diffusion method)

Bacterial strains	Zone of inhibition in mm						
	Methanol extract						Sterile water
	Andrographis paniculata			*Melia azadirach L.*			
	25µl	50µl	100µl	25µl	50µl	100µl	
Pseudomonas aeruginosa	22	13	-	11	10	_	-
Vibrio cholerae	14	12	11	13	14	18	-
Escherichia coli	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-
Salmonella typhi	12	12	8	13	_	10	-
Staphylococcus aureus	21	10	8	12	14	14	-

Table 3. Antifungal activity of the methanol extract of *Andrographis paniculata* and *Melia azadirac.L* (Agar Disc diffusion method)

Fungal strains	Zone of inhibition in (mm)		
	Methanol extract		Sterile water
	Andrographis paniculata	*Melia azadirach L.*	
	100µl	100µl	100 µl
Trycophyton rubrum	11	10	-
Aspergillus niger	-	-	-
Aspergillus fumigatus	24	16	-
Candida albicans	12	14	-

A.paniculata and *M.azadirach.L* showed potential antifungal activity against Aspergillus *fumigatus*, the zone of inhibition level of 24mm and 14mm respectively (Table 3). Three spots were obtained from the methanol extracts of *A.paniculata* with the RF value range (0.43, 0.38 and 0.16) and two spots obtained from the *M.azadirach* with the RF value range 0.46, 0.27 when eluted with the solvent system EA: Methanol (16:22) using TLC technique

Conclusion

The present investigation explored the antimicrobial potential of the medicinal plant *A.paniculata* and *M. azadirach* L. against the clinical bacterial and fungal pathogens. However, remarkable activity was noticed against two bacterial strains *viz.*, *Klebsiella pneumoniae* and *Vibrio cholerae*. These are the major harmful pathogens from the clinical background affecting the human beings. As the curde extracts of antimicrobial activity, separation of an individual compounds from the crude extract of this plants will be resulted in the development of a novel antibiotic.

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